Preliminary and Short Report

QUANTITATIVE ESTIMATION OF CHITIN IN TRICHOPHYTON MENTAGROPHYTES AND THE *IN VITRO* EFFECT OF CHITINASE*

HERBERT N. PRINCE, PH.D.†

Chitin, protein, callose, pectin, and cellulose have long been recognized as constituents of fungal cell walls (1), but only chitin has been demonstrated in the cell walls of dermatophytes (2). This substance alone comprised the continuous skeletal material of the cell walls of species of *Trichophyton* and *Epidermophyton*; however, quantitative measurements were not made. We are concerned here with the quantitative estimation of chitin in the mycelium of *Trichophyton mentagrophytes* (WωT 609). In addition, observations are presented on the effect of the enzyme chitinase on the cell structure and growth of this organism

T. mentagrophytes was grown, harvested, and the lipids extracted as previously described (3). The fat-free residue was then refluxed in 10 per cent NaOH solution (three 700 ml. portions) for 31 hours, after which time the mycelium appeared as a tan-white mass with the consistency of cooked oatmeal. The alkaline-resistant mass was washed with hot water until neutral. Cytoplasm was completely removed by this treatment as evidenced by unstainability of the hyphal cells with crystal violet and sudan black. Skeletal cells and hyphae so obtained appeared microscopically to have the same size and shape as living cells.

The washed cell wall fraction (N, 6.2 per cent; calculated for chitin, 6.9 per cent) was hydrolyzed with 6N HCl according to the method of Falcone and Nickerson (4). The hydrolyzate was filtered through charcoal and reduced to dryness by distillation *in vacuo*. The residue was dissolved in 5 ml. of water and the resulting light orange solution chromatographed on Whatman no. 1 paper (phenol:water), the water-rich stationary phase containing NH_3 and KCN. Glucosamine was detected in this procedure according to the method of Partridge (5); sugars were detected with acidic aniline phthalate (6); amino acids were developed with ninhydrin. Controls of 20 amino acids, glucosamine-HCl, N-acetyl glucosamine, glucose, galactose, mannose, ribose, xylose, maltose, cellobiose, lactose, sucrose, glucuronic and galacturonic acids were spotted. Spots with R_f values corresponding only to glucosamine·HCl were detected. Acetone-insoluble crystals precipitated from the HCl hydrolyzate and recrystallized from ethanol melted at 106–109° C (glucosamine, 110° C.)

The alkaline-resistant cell wall fraction, therefore, consisted essentially of chitin and represented 3.2 per cent of the moist mycelium (14.4 per cent by dry weight). Comparisons with the data of Blumenthal and Roseman (7) indicated that this strain of *T. mentagrophytes* contained roughly the same amount of chitin as *Asperigillus flavus*, *Aspergillus niger*, *Neurospora tetraspora*, and *Rhizopus nigricans*.

An experiment was performed to determine the effect of chitinase on the living cells of the test organism. The source of chitinase was an unidentified streptomycete, isolated from New Jersey soil, capable of utilizing chitin as a sole source of carbon and nitrogen. Hyphal strands and macroconidia (thrice-washed in saline) were suspended in a mineral salts agar containing 0.3 per cent purified powdered chitin. Plates were cross-streaked with a washed suspension of the chitinolytic streptomycete.

Dissolution of the chitin around the streptomycete colonies was evident after 4 days of incubation at room temperature. Microscopic examination of pieces of agar cut from these clear zones revealed intact branching hyphae and germinating macroconidia. Similar signs of growth were seen in the opaque non-dissolved areas, indicating that the organism itself was able to utilize chitin. After 10 days the dermatophyte erupted over the entire surface appearing even on top of the streptomycete colonies. Growth of *Trichophyton* was always heaviest in the clear areas suggesting utilization of soluble products of digestion.

It was concluded that extracellular chitinase in no way affected the structure or viability of *Trichophyton mentagrophytes*. Since chitin alone comprised the continuous skeletal material of this organism, other substances within or surrounding

* From the Research Department, Wallace and Tiernan, Inc. Belleville, N. J.
† Present address: Chemotherapy Department, Hoffman-LaRoche, Inc., Nutley, New Jersey.
Received for publication February 4, 1960.

the cell wall apparently shielded this substance from the action of the enzyme.

REFERENCES

1. Foster, J. W.: The Chemical Activities of Fungi. New York, Academic Press, 1949.
2. Blank, F.: The chemical composition of the cell walls of dermatophytes. Biochim. et Biophys. Acta., **10:** 110–113, 1953.
3. Prince, H. N.: Total lipid of Trichophyton mentagrophytes. J. Bact., **79:** 154, 1960.
4. Falcone, G. and Nickerson, W. J.: Cell-wall mannanprotein of baker's yeast. Science, **124:** 272–273, 1956.
5. Partridge, S. M.: Filter-paper partition chromatography of sugars. Biochem. J., **42:** 238–250, 1948.
6. Gaillard, B. D. E.: Use of unneutralized hydrolyzates in paper chromatography of sugars. Nature, **171:** 1160, 1953.
7. Blumenthal, H. J. and Roseman, S.: Quantitative estimation of chitin in fungi. J. Bact., **74:** 222–224, 1957.